The results from this new work are important not only because of the possible therapeutic implications for Gaucher disease but also because they highlight the fundamental difference between transport to lysosomes mediated by LIMP-2 and by the mannose 6-phosphate receptor (Figure 1). Recognition of the mannose 6-phosphate carbohydrate modification on lysosomal hydrolases by the mannose 6-phosphate receptor occurs in the *trans*-Golgi network, whereas LIMP-2 recognition of βGC can take place in the ER and is apparently carbohydrate independent. Lysosomal hydrolases dissociate from the mannose 6-phosphate receptor in late endosomes as this receptor is not present in lysosomes, whereas LIMP-2 is present in lysosomes, suggesting that dissociation of βGC from LIMP-2 may occur in the lysosome itself. It is not clear whether LIMP-2 might mediate sorting of other proteins to the lysosome. It is also not clear whether LIMP-2 delivers newly synthesized proteins, endocytosed proteins, or both to lysosomes. However, the low levels of LIMP-2 found on the plasma membrane suggest that it may act primarily inside the cell.

These results provide yet another example of where the study of a lysosomal storage disease has provided insights into the fundamental mechanisms controlling protein sorting in cell biology. But what insights into Gaucher disease do these findings provide? One intriguing possibility raised by the authors is that some patients with Gaucher-like phenotypes might have mutations in LIMP-2 that might prevent proper sorting of βGC. Further studies of LIMP-2-deficient mice will certainly provide insights into this possibility. The current studies also open up new ways in which to understand the loss-of-function mutations in βGC found in patients with Gaucher disease in terms of their recognition and transport by LIMP-2. Furthermore, by identifying the machinery that governs the secretion of βGC, these studies may provide ways to improve production of βGC, which is used therapeutically to treat patients with Gaucher disease. But perhaps most importantly these studies highlight the enormous synergy between studies of genetic diseases and our understanding of cell biology.

REFERENCES

de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955). Biochem. J. *60*, 604–617.

Kornfeld, S. (1990). Biochem. Soc. Trans. *18*, 367–374.

Reczek, D., Schwake, M., Schroder, J., Hughes, H., Blanz, J., Jin, X., Brondyk, W., Van Patten, S., Edmunds, T., and Saftig, P. (2007). Cell, this issue.

Rijnboutt, S., Aerts, H.M.F.G., Geuze, H.J., Tager, J.M., and Strous, G.J. (1991). J. Biol. Chem. *266*, 4862–4868.

Gene Silencing CUTs Both Ways

Nick Proudfoot[1,*] and Monika Gullerova[1]
[1]Sir William Dunn School of Pathology, South Parks Rd., OX1 3RE, University of Oxford, Oxford, UK
*Correspondence: nicholas.proudfoot@path.ox.ac.uk
DOI 10.1016/j.cell.2007.11.003

There is extensive transcription throughout the eukaryotic genome resulting in both antisense transcripts from coding regions and cryptic unstable transcripts (CUTs) from intergenic regions. In this issue, Camblong et al. (2007) demonstrate in the budding yeast that antisense transcripts, if stabilized by exosome impairment, are able to mediate gene silencing via the recruitment of histone deacetylases.

Silencing is a critical feature of gene regulation in both unicellular and higher eukaryotes. In the fission yeast *Saccharomyces pombe*, gene silencing has been shown unexpectedly to involve the RNA interference (RNAi) pathway and in particular the RNase III enzyme Dicer. In higher eukaryotes, Dicer is responsible for cutting up double-stranded RNA (dsRNA) into either small-interfering RNAs (siRNAs) or microRNAs that then regulate mRNAs through their incorporation into the RISC complex. This RNA-primed complex targets specific mRNAs, either promoting their degradation or inhibiting their translation. Instead, in *S. pombe*, Dicer is associated with a chromatin-silencing complex (RITS) that is recruited to regions of heterochromatin caught in the act of low-level transcription from both template strands thereby generating dsRNA. Indeed, most other higher eukaryotes share this RNAi-induced gene-silencing pathway (Grewal and Jia, 2007). However, the budding yeast *S. cerevisiae* seems bereft of RNAi-mediated gene regulation, as it lacks Dicer, Argonaute proteins, and RNA-dependent RNA polymerase. Yet, gene silencing still occurs in *S. cerevisiae*. So how is it achieved?

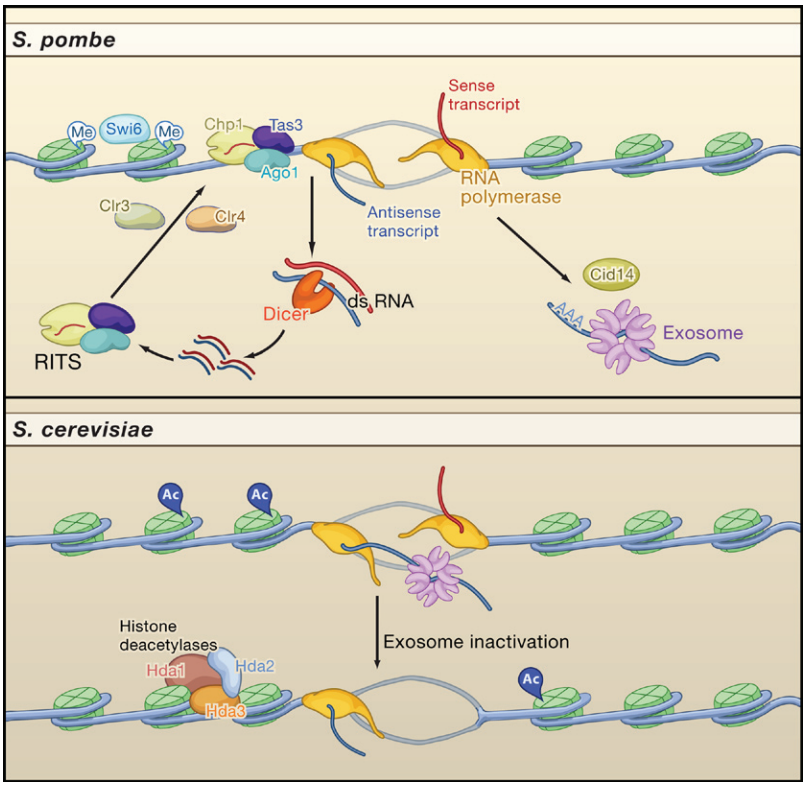

Figure 1. Parallel Mechanisms for Gene Silencing in Yeast

(Top) In *S. pombe*, heterochromatin is maintained through synthesis of double-stranded RNA (dsRNA) generated through low-level transcription from both DNA strands by RNA polymerase II with subsequent amplification by RNA-dependent RNA polymerase. Double-stranded RNA is degraded either by the exosome or Dicer. Dicer generates siRNAs that are incorporated into the RITS complex, which then targets homologous chromatin resulting in gene silencing by histone tail deacetylation and methylation followed by recruitment of Swi6. (Bottom) In *S. cerevisiae*, specific genes generate antisense transcription, which is normally suppressed by exosome-mediated degradation. However, if exosome activity is limited, antisense cryptic unstable transcripts (CUTs) accumulate. This causes recruitment of histone deacetylases to nearby chromatin, resulting in gene silencing. Swi6 is an Hp1 homolog; Clr3 and Hda1-3 are histone deacetylases; Clr4 is a histone methyltransferase; Cid14 is an exosome-activating polyA polymerase.

The study by Camblong et al. (2007) in this issue provides new exciting clues. They show that the stabilization of antisense RNAs in *S. cerevisiae* can lead to the recruitment of histone deacetylases to inhibit sense transcription (Figure 1).

In *S. cerevisiae*, transcriptional interference occurs if transcription across one gene fails to terminate. This results in read-through transcription, which causes downregulation of the next gene along the chromosome (Shearwin et al., 2005). If the downstream gene faces in the same direction (in tandem) then its promoter may be blocked by the read-through transcription (Greger et al., 2000). Such an interference process occurs naturally in *S. cerevisiae* for the *SER3* gene where an upstream promoter generates a cryptic unstable transcript (CUT) reading into *SER3* that then regulates the activity of the *SER3* gene by blocking transcription initiation (Martens et al., 2004). Alternatively, if the read-through transcript extends into a convergent downstream gene then polymerases may collide with each other causing an elongation block for both transcripts (Prescott and Proudfoot, 2002). This mechanism is adopted by the *IME4* gene, which controls the onset of meiosis in diploid cells. Here, the gene appears to exist as two wholly overlapping transcription units. When the antisense gene is active, as occurs in haploid cells, then *IME4* transcripts are switched off. However in diploid cells of mixed a/α mating type, an a/α repressor protein is synthesized that blocks the antisense promoter. This allows *IME4* transcription to take off (Hongay et al., 2006) and consequently allows the a/α diploid cell to proceed to meiotic division. Exactly how antisense transcription blocks sense transcription is unresolved.

The efforts of Camblong et al. provide new insight into how antisense transcription can block gene expression in *S. cerevisiae*. Like many important discoveries this story begins with a serendipitous finding. It was noticed that transcription from the *PHO84* gene progressively decreases if yeast colonies are artificially aged on minimum medium plates or in cultures left in the fridge for a few weeks. Other genes including those neighboring *PHO84* are unaffected by this ill treatment, suggesting that *PHO84* possesses a specific gene-silencing mechanism that is promoted under stress conditions. Indeed, a low-level antisense CUT is found to accumulate at the expense of *PHO84* mRNA, and (as with other CUTs) knockdown of the RNA degradation apparatus, in particular nuclear exosome components (Wyers et al., 2005), increases levels of the antisense CUT to further impair *PHO84* sense transcription. The authors then show that binding of the exosome (Rrp6) to chromatin associated with the antisense CUTs is diminished in aged cells and this presumably is the cause of the age-associated effect.

So how does the antisense *PHO84* transcript silence the gene without using RNAi's trickery? The authors resolve this question by showing that histone deacetylases (Hda1-3) are specifically recruited to antisense-repressed *PHO84* resulting in inactivation of the *PHO84* promoter region due to loss of histone acetylation. In other words, *PH084* in aged *S. cerevisiae* acquires a heterochromatin epigenetic mark just like the RNAi-induced silencing mechanism characterized in *S. pombe.* In fact, the situation in *S. pombe* may be much closer to *S. cerevisiae* than would be thought at first glance. *S. pombe* uses not only the RITS complex to induce hetero-

chromatin but also an independent process involving the recruitment of histone deacetylases (HDACs). Furthermore, this latter process appears to be the dominant gene-silencing pathway used for the region of the genome involved in the mating type switch (Yamada et al., 2005). Also, dissection of the RNAi mechanism of gene silencing in *S. pombe* shows that the exosome and in particular an associated polyA polymerase (Cid14) are required for gene silencing (Buhler et al., 2007). The fact that silencing of the *PHO84* gene involves both the exosome (Rrp6) and HDACs strikingly parallels these aspects of silencing in *S. pombe*. Indeed, in using both RNAi and HDAC-associated pathways for gene silencing, *S. pombe* demonstrates its evolutionary position at center stage between *S. cerevisiae* and mammals.

As with all advances, new answers raise new questions. First, it is important to know how many other genes in *S. cerevisiae* besides *PHO84* are subject to a similar process of stress-induced gene silencing. Clearly, expression array analysis is called for here. Also, from an evolutionary perspective it is interesting that for *S. pombe* and also possibly in plants, RNAi-induced gene silencing is the predominant use of RNAi. In contrast, in higher eukaryotes, RNAi appears to primarily downregulate mRNA expression and inhibit translation efficiency in the cytoplasm via the actions of siRNAs and microRNAs. Apparently, there are no clear protein homologs in *S. cerevisiae* of the well-defined RNAi apparatus in *S. pombe* and higher eukaryotes. However, some surprises may await us that may reveal a more unifying mechanism for all eukaryotic gene silencing. Clearly, the rapid degradation of newly synthesized CUTs, both sense and antisense, is a key aspect of gene silencing in eukaryotes.

REFERENCES

Buhler, M., Haas, W., Gygi, S.P., and Moazed, D. (2007). Cell *129*, 707–721.

Camblong, J., Iglesias, N., Fickentscher, C., Dieppois, G., and Stutz, F. (2007). Cell, this issue.

Greger, I.H., Aranda, A., and Proudfoot, N. (2000). Proc. Natl. Acad. Sci. USA *97*, 8415–8420.

Grewal, S.I., and Jia, S. (2007). Nat. Rev. Genet. *8*, 35–46.

Hongay, C.F., Grisafi, P.L., Galitski, T., and Fink, G.R. (2006). Cell *127*, 735–745.

Martens, J.A., Laprade, L., and Winston, F. (2004). Nature *429*, 571–574.

Prescott, E.M., and Proudfoot, N.J. (2002). Proc. Natl. Acad. Sci. USA *99*, 8796–8801.

Shearwin, K.E., Callen, B.P., and Egan, J.B. (2005). Trends Genet. *21*, 339–345.

Wyers, F., Rougemaille, M., Badis, G., Rousselle, J.C., Dufour, M.E., Boulay, J., Regnault, B., Devaux, F., Namane, A., Seraphin, B., et al. (2005). Cell *121*, 725–737.

Yamada, T., Fischle, W., Sugiyama, T., Allis, C.D., and Grewal, S.I. (2005). Mol. Cell *20*, 173–185.

RecBCD: The Supercar of DNA Repair

Dale B. Wigley[1,*]

[1]Cancer Research UK Clare Hall Laboratories, The London Research Institute, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3LD, UK

*Correspondence: dale.wigley@cancer.org.uk

DOI 10.1016/j.cell.2007.11.004

The DNA helicase RecBCD pauses when it reaches recombination hotspots known as Chi sites and then proceeds at a slower speed of translocation than before Chi recognition. Reporting in this issue, Spies et al. (2007) now show that this reduction in translocation velocity occurs when RecBCD changes which of its two motor subunits is in the lead.

Motor car fans will be familiar with the Bugatti Veyron, until a few weeks ago the world's fastest supercar. The Veyron is an astonishing engineering achievement powered by the fusion of two V8 engines to create a single W16 quad-turbocharged motor that produces 1001 bhp and is capable of speeds of over 250 mph. Many readers of *Cell*, however, may be more familiar with the "supercar" of DNA repair, RecBCD. This enzyme is responsible for initiating repair of double-strand breaks in many bacteria. Like the Veyron, RecBCD contains two engines (the RecB and RecD helicase motor subunits; see Figure 1) that are capable of driving the complex along DNA at over 1000 base pairs per second. The RecB and RecD motors are each powered by hydrolysis of ATP, the combination consuming two ATP molecules per base pair. Significantly, RecBCD is more cleverly engineered than the Veyron because the two motors can work independently. In fact, in work presented in this issue, Spies et al. (2007) show that following the recognition of recombination hotspots called Chi (crossover *h*otspot *i*nstigator) sites, RecBCD is able to switch which of its two motors takes the lead and thereby regulate the translocation velocity of the complex.